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Agilent Technologies

LC/MS Application Note

Number 9
November 2003

Femtogram Sensitivity of the Antipsychotic Drugs Raclopride and Ritanserin Using a Varian 1200L LC/MS/MS

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Introduction

Ritanserin (Tisterton) is a serotonin-2 antagonist drug shown to be effective in the treatment of anxiety and depression[1] while Raclopride acts as a dopamine D2 receptor antagonist.[2] These two compounds were used to demonstrate low-level sensitivity of the Varian 1200L LC/MS/MS in selected reaction monitoring mode (SRM). Studies have shown LC/MS to be a useful tool in the quantitation of low levels of Raclopride.[3]

Instrumentation

- Varian 1200L LC/MS/MS equipped with ESI source

Materials and Reagents

- Ritanserin ordered from Sigma-Aldrich Corp., Catalog Number R-103
- S-(-)-Raclopride ordered from Sigma-Aldrich Corp., Catalog Number R-121
- All other chemicals are reagent grade or HPLC grade

Sample Preparation

Milligram quantities were prepared and dissolved in a 50:50 mixture of acetonitrile/water with 0.1% formic acid. Serial dilutions were made from each stock solution prior to flow injection analysis (FIA). Samples were injected into a mobile phase of 50:50 acetonitrile/0.1% formic acid which was pumped directly into the mass spectrometer at 0.2 mL/min. A 5 µL loop was used for the injections and the injections were repeated five times for each concentration level.

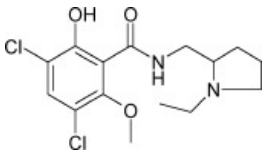
MS Parameters

Collision Gas	2.2 mTorr Argon
API Drying Gas	20 psi at 325 °C
API Nebulizing Gas	55 psi
Scan Time	0.25 sec
SIM Width	0.7 amu
Needle	5000V
Shield	600V
Capillary	55V
Detector	1500V

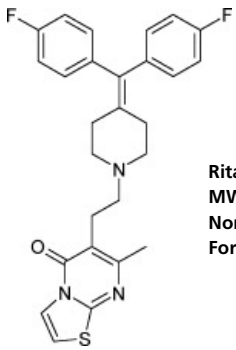
Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
Ritanserin	478	193	26.5
Raclopride	347	112	22

Compound Structures



Raclopride
MW = 347.24
Nominal Mass = 346
Formula = $C_{15}H_{20}Cl_2N_2O_3$



Ritanserin
MW = 477.58
Nominal Mass = 477
Formula = $C_{27}H_{25}F_2N_3OS$

Results and Discussion

Raclopride – Five replicates at five different concentration levels were run. FIA injections of Raclopride at its lowest detectable limit, 100 pg/mL (500 fg on-column), is shown in Figure 1. At the lowest level, the S/N ratio is approximately 10:1 for the raw data. Also, a plot at a higher concentration of 10 ng/mL is included for comparison (Figure 2). The calibration curve in Figure 3 shows the average area of the five replicates plotted against the amount injected.

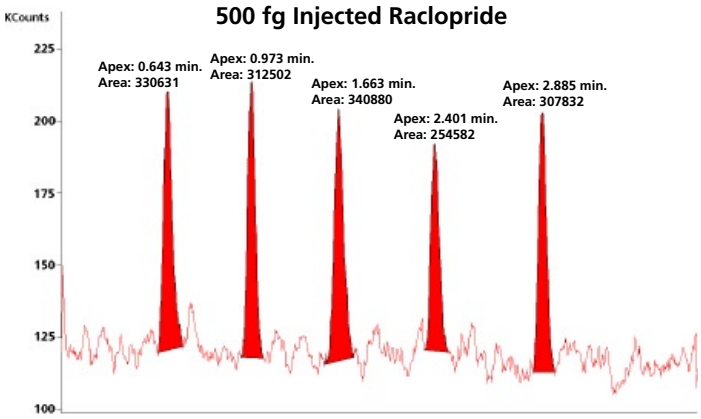


Figure 1. FIA of 500 fg Raclopride at low concentration shows good reproducibility with a 10.7% RSD and sensitivity S/N approximately 10:1 (RMS).

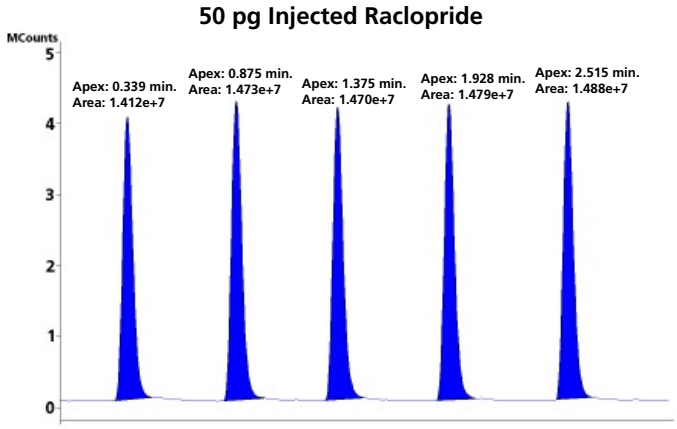


Figure 2. FIA of 50 pg Raclopride at a higher concentration of 10 ng/mL shows good reproducibility with a 2.1% RSD.

Ritanserin – Five replicates at five different concentration levels were run. FIA injections of Ritanserin at its lowest detectable limit, 10 pg/mL (50 fg on-column), is shown in Figure 4. At a concentration 10 times lower than Raclopride, Ritanserin is easily detected. With a S/N ratio of more than 20:1 at 10 pg/mL, it should be possible to achieve even lower limits of detection for Ritanserin.

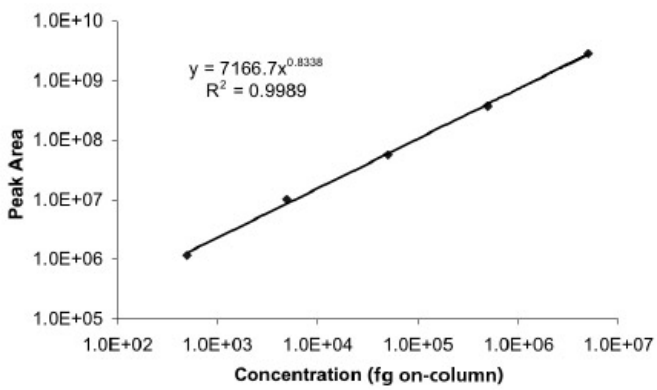


Figure 3. The calibration curve for Raclopride shows excellent linearity over five orders of magnitude of concentration.

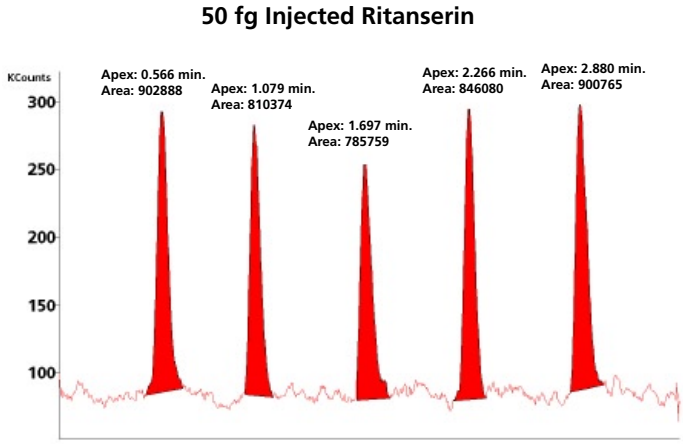


Figure 4. With FIA of 50 fg Ritanserin at very low concentration, the Varian 1200L LC/MS attains excellent S/N >20:1 (RMS) and a 6.2% RSD.

Conclusion

The ability to accurately detect low levels of analytes is an essential requirement for many pharmaceutical, toxicological, and clinical applications. The data presented in this application note illustrates that the Varian 1200L triple quadrupole mass spectrometer is capable of achieving very reproducible results at femtogram levels for compounds such as Ritanserin and Raclopride.

References

1. http://www.books.md/R/dic/ritanserin.php
2. http://www.online-medical-dictionary.org/?q=Raclopride
3. Forngren, B.H. Liquid Chromatography-Mass Spectrometry in the Analysis of Radiolabelled Compounds for Positron Emission Tomography. Acta Universitaris Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 586. 56pp. Uppsala. ISBN 91-554-4810-0

These data represent typical results.
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